Conference Paper

# Methods and Applications of Label-Free Cell-Based Systems

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## Abstract

Label-free monitoring of living cells is used in various applications such as drug development, toxicology, regenerative medicine or environmental monitoring. The most prominent methods for monitoring the extracellular acidification, oxygen consumption, electrophysiological activity and morphological changes of living cells are described. Furthermore, the intelligent mobile lab (IMOLA) – a computer controlled system integrating cell monitoring and automated cell cultivation – is described as an example of a cell-based system for microphysiometry. Results from experiments in the field of environmental monitoring using algae are presented. An outlook toward the development of an organ-on-chip technology is given.

**Keywords:** microphysiology, cell-based assay, microsensors, environmental monitoring, toxicology, drug development

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## 1. Introduction

To allow the continuous and therefore dynamic monitoring of the vitality of living cells, the method of investigation must not interact with the living probe. Therefore, labels or markers which bind to receptors (e.g. the G-protein coupled receptor (GPCR)) are prohibited. Beside methods based on optical analysis [1] a variety of mainly electrochemical methods are available. Those methods address e.g. the extrusion of $H_3O^+$, cellular consumption of dissolved oxygen, electrophysiological activity and changes in the dielectricity of whole cells or the transmembrane flux of ions. Extrusion of $H_3O^+$ (extracellular acidification) are addressed by light addressable potentiometric sensors (LAPS) [2], ion sensitive field effect transistors (ISFETs) [2,3] or with metal-oxide based sensors [4]. The main principle to measure dissolved oxygen consumption (cellular respiration) is amperometry [5,6]. Electrophysiological activity or action potentials can be measured with micro-electrode arrays (MEAs) [2] while changes in dielectricity (e.g. beating of cardiomyocytes) or the transmembrane flux of ions (e.g. lysis of the cellular membrane) are addressed with impedance measurement [7,8] .

The Cytosensor Microphysiometer is a monitoring system based on the LAPS [9]. It gained acceptance for eye irritation assessment in 2009 [10]. The Bionas DiscoveryTM 2500 system [11] incorporates ISFETs, amperometric microsensors and impedance structures – manufactured in silicon technology - for live cell monitoring. Different commercial systems for electrophysiological monitoring with MEAs are available

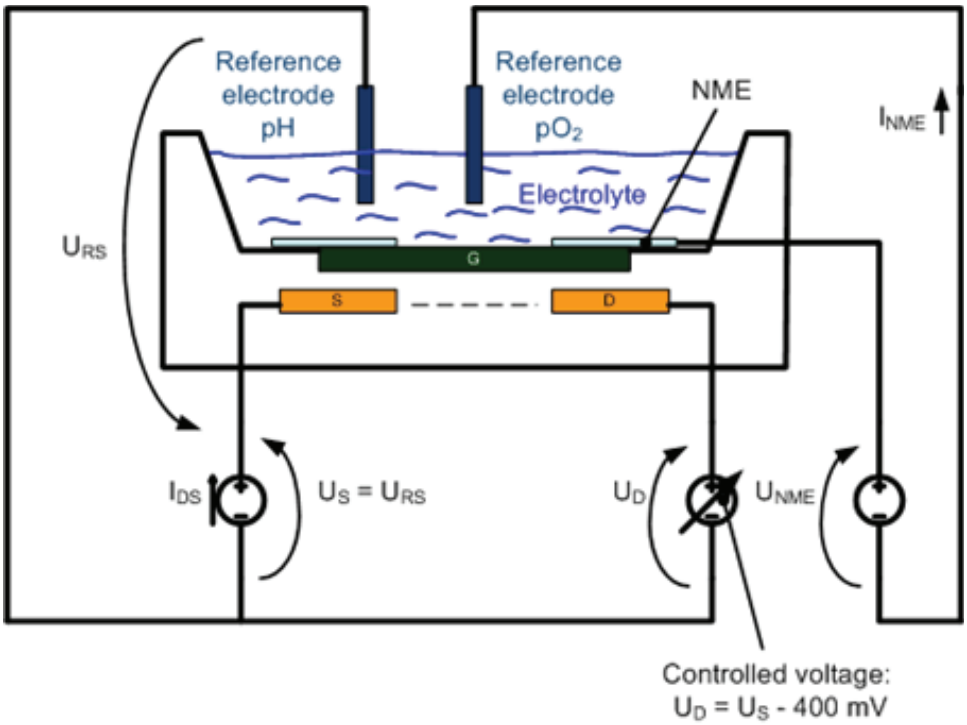


**Figure** 1: Principle of the CVFET. The gate electrode of a conventional ISFET is surrounded with a noble metal electrode where a cyclic potential is applied.

from Multichannel Systems MCS GmbH (Reutlingen, Germany) or Neuroproof GmbH (Rostock, Germany). Various systems for impedance monitoring were described by Schwarzenberger et al. [8]. A system which combines impedance measurement with opto-analytical methods is the intelligent microplate reader (IMR) from HP Medizintechnik GmbH (Oberschleißheim, Germany) [12].

## 2. Materials and Methods

The principle of the ISFET was extended toward the monitoring of dissolved oxygen [13] and further developed toward a cyclic voltammetry field effect transistor (CVFET) [14]. Figure 1 shows the principle of the CVFET. The pH-sensitive gate electrode of the ISFET is surrounded by a noble metal electrode (NME) where cyclic voltammetry vs. an external silver/silver-chloride reference electrode is performed. Whenever an electrochemical reaction at the NME provokes production of $H_3O^+$ or OH- the so-caused change in pH is also detected at the ISFET. That allows a more detailed interpretation of the electrochemical process [14].

The 6xIMOLA-IVD (Figure 2) is a turn-key ready system for automated label-free analysis of living cells [15]. The system is mounted in an incubator to allow monitoring at 37°C. It incorporates BioChips for simultaneous monitoring of extracellular acidification, cellular respiration and changes in cellular morphology. To enable automated analysis with defined standard operating procedures (SOPs) a fluidic system including switch valves, a computer controlled peristaltic pump and a software application for control and data acquisition is integrated. To be able to perform replicates, positive,

**Figure** 2: 6xIMOLA-IVD system. Six stations with BioChips are each extended with fluidic modules to switch between 4 different media flasks (24 channels). The measurement is arranged in an incubator to keep the living cells at 37°C. A computer takes care of the control and data acquisition.

negative and a blank control six parallel modules are set up. Since each module can be programmed to switch between four media flasks, 24 channels are possible.

## 3. Results

The capability of the 6xIMOLA-IVD for applications in fields as toxicology, cancer research or environmental monitoring was shown [15]. Figure 3 shows an example where the photosysnthetic activity of algae is inhibited during the presence of the xenobiotic Metamitron [16]. Recent experiments were performed on the algae chlorella kessleri with samples from Indonesian palm oil [17]. First results where the photosynthetic activity of the algae under the influence of samples from palm oil was monitored indicate that the algae increase their metabolism due to the availability of palm oil.

## 4. Discussion

Various types of microsensors are available for label-free monitoring of parameters of living cells. Integration of those sensors into a computer controlled monitoring system including a fluidic system allows long-term investigation of living cells and their interaction with changes in their microenvironment. Applications in the field of toxicology with cells in monolayer were performed by different groups. Results from the field of environmental monitoring using cells in suspension were presented. New developments are heading toward the investigation of 3D cell structures to allow the modeling of organ functions with label-free cell based assays [18].

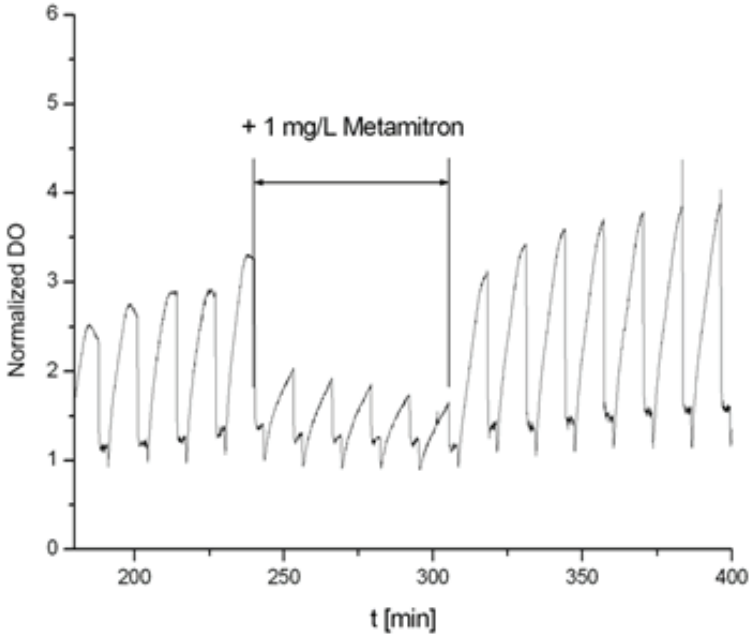


**Figure** 3: Photosynthethic activity of the algae chlorella kesleri. The photosynthetic activity is inhibited during the presence of Metamitron.

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